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#### 14. ABSTRACT

**Purpose:** The purpose of this study investigated the antimicrobial efficacy of ultraviolet light (UVL) with and without traditional antibiotic therapy over a 14-day study duration. The objective was to assess microbial load of the implanted materials in experimental and control groups.

**Design:** Experimental, prospective study.

**Methods:** Eighty Sprague-Dawley rats were randomly assigned to one of four groups: Group 1, (Ceftriaxone); Group 2 (UVL only); Group 3, (UVL plus Ceftriaxone); or Group 4 (no treatment). A 2 cm incision was made and *staphylococcus aureus* inoculated Copa Foam was inserted. Samples were collected on day three, five, ten, and fourteen. Groups 1 and 3 were administered Ceftriaxone 100 mg/Kg on day 0 and day 1. Groups that received UVL received the light one inch from the wound site for 180 seconds.

**Sample:** Eighty male, Sprague Dawley Rats (300-400g) were used.

**Analysis:** Analysis of Variance (ANOVA) and repeated ANOVA (RANOVA) were used.

**Findings:** An ANOVA indicated no significant differences in the weights of the rats by group indicating that the groups were equivalent on this variable ( $p > 0.05$ ). RANOVA determined if there were significant differences in microorganism counts by groups over time. The microorganism count for the Ceftriaxone Group was less than the UVL Group on days 3, 5, and 10 ( $p < 0.05$ ). There was no significant differences in count between the UVL group and the no treatment groups at any time ( $p > 0.05$ ). There were no differences between the Ceftriaxone only group and the combination of Ceftriaxone + UVL at any time ( $p > 0.05$ ).

**Implications for Military Nursing:** The data show that UVL had little effect on microorganism count. By the fourteenth day, all groups increased microorganism count indicating that antibiotic treatment needs to continue.

#### 15. SUBJECT TERMS

Nano Germicidal, rats, ultraviolet light, Ceftriaxone

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**TriService Nursing Research Program Final Report Cover Page**

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Date

5 Oct 2016

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**TSNRP Research Priorities that Study or Project Addresses****Primary Priority** Identify the primary research priority addressed in the study or project.

Force Health Protection:	<input type="checkbox"/> Fit and ready force <input type="checkbox"/> Deploy with and care for the warrior <input type="checkbox"/> Care for all entrusted to our care
Nursing Competencies and Practice:	<input type="checkbox"/> Patient outcomes <input type="checkbox"/> Quality and safety <input type="checkbox"/> Translate research into practice/evidence-based practice <input type="checkbox"/> Clinical excellence <input type="checkbox"/> Knowledge management <input type="checkbox"/> Education and training
Leadership, Ethics, and Mentoring:	<input type="checkbox"/> Health policy <input type="checkbox"/> Recruitment and retention <input type="checkbox"/> Preparing tomorrow's leaders <input type="checkbox"/> Care of the caregiver
Other: (specify)	<input type="checkbox"/>

**Secondary Priority (**

Force Health Protection:	<input type="checkbox"/> Fit and ready force <input type="checkbox"/> Deploy with and care for the warrior <input type="checkbox"/> Care for all entrusted to our care
Nursing Competencies and Practice:	<input type="checkbox"/> Patient outcomes <input type="checkbox"/> Quality and safety <input type="checkbox"/> Translate research into practice/evidence-based practice <input type="checkbox"/> Clinical excellence <input type="checkbox"/> Knowledge management <input type="checkbox"/> Education and training
Leadership, Ethics, and Mentoring:	<input type="checkbox"/> Health policy <input type="checkbox"/> Recruitment and retention <input type="checkbox"/> Preparing tomorrow's leaders <input type="checkbox"/> Care of the caregiver
Other:	<input type="checkbox"/>

## **Progress towards Achievement of Specific Aims of the Study or Project**

### **Findings related to each specific aim, research or study questions, and/or hypothesis:**

The aim of experiment one was to determine the effect of ultraviolet light (UVL) with and without traditional therapy over a 14-day study duration. Eighty pathogen-free, male, Sprague Dawley Rats weighing between 300-400 grams were purchased and were quarantined for seven days. To help reduce experimental variability, rats were procured from the same vendor, were approximately the same size, weight, were same gender (male), and were the same species. The rationale for using male rats was to avoid any potential hormonal effects.

Surgical site infection is one of the major sources of postoperative complications; therefore, prevention or reduction in wound infections is a top priority for quality patient care. Infections delay the healing process of wounds subsequently adding to increased costs and deaths. Treatments using ultraviolet germicidal light (UVL) have been purported to improve the wound healing process by destroying microorganisms. Germicidal light is defined as short-wave ultraviolet energy (100 to 280 nanometers) and may be highly effective method for destroying microorganisms relative to wounds.

Infections acquired by patients during the course of receiving treatment for other conditions are common. Each year wound infections affect more than two million patients or 5-10% of all hospitalized patients.<sup>1,2</sup> Some of these infections are acquired by the transmission from healthcare workers while performing their duties.<sup>3</sup> Such infections are associated with approximately 90,000 deaths each year.<sup>4,5</sup> An infection is considered to be an surgical associated infection when it occurs at the site of surgery within 30 days of an operation or within 1 year of an operation if a foreign body is implanted as part of the surgery.<sup>6</sup> Infections are particularly problematic when associated with multi-drug resistant (MDR) microorganisms. The spread of MDR bacteria creates a serious threat, particularly in intensive care units (ICUs), where it is estimated up to 30% of hospital acquired infections occur.<sup>7</sup> Antibiotic use is elevated in ICUs, antibiotic use is increased, opportunities for pathogen transmission are increased, and a large proportion of patients are immunocompromised resulting in increased susceptibility to infection.<sup>8-12</sup> Causative microorganisms often include multi-resistant pathogens such as gram-positive methicillin-resistant *staphylococcus aureus* (MRSA) and/or gram-negative *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter* species.<sup>13-16</sup> Infections are associated not only with increased morbidity but also with mortality. In addition to significant morbidity and mortality, infections are associated with prolonged hospital stays,<sup>17</sup> higher healthcare costs,<sup>18-20</sup> and add approximately \$4.5 billion to the national health bill.<sup>18,21-23</sup> Evans and colleagues investigated the cost of infections.<sup>24</sup> Patients with resistant bacterial infection had higher severity of illness, higher median hospital costs (\$80,500 vs. \$29,604,  $p < .0001$ ), higher median antibiotic costs (\$2,607 vs. \$758,  $p < .0001$ ), longer median hospital length of stay (29 vs. 13 days,  $p < .0001$ ), and a longer median ICU length of stay (13 days vs. 1 day,  $p < .0001$ ).<sup>24</sup>

### **Pathophysiology of Wounds**

Numerous complex interrelated biological process affect wound healing.<sup>25</sup> Interventions that that prevent or facilitate recovery result in better outcomes and shortest time to healing. Infection and healing occur in stages.<sup>26</sup> The inflammatory phase commences as soon as tissue integrity is

disrupted by injury, initiating the coagulation cascade to limit bleeding.<sup>27</sup> Platelets are the first of the cellular components that aggregate to the wound resulting in degranulation and the release of cytokines and growth factors. These cytokines are critical in the healing processes and culminates in the recruitment of wound healing through leukocytes and stimulation of fibroblasts and epithelial cells. Specifically, the cytokines include platelet derived growth factor, insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), and fibroblast growth factor (FGF).<sup>28</sup> The resulting exudate contains red blood cells, neutrophils, macrophages, and plasma proteins, proteins and fibrin strands.<sup>27</sup> The major job of the macrophages is to scavenge for infection and to serve as a central component releasing cytokine of wound healing process.<sup>29</sup> During the proliferative phase, cells such as fibroblasts, epithelial cells, and vascular endothelial cells migrate to the site of injury and begin to proliferate. The cytokines involved in this phase stimulate angiogenesis and epithelial cell and fibroblast proliferation.<sup>30</sup> The basal cells aggregate at the wound, and within 48 hours, the entire wound is epithelialized.<sup>31</sup> With infection, progressive destruction of previously healthy tissue occurs. It is critical to implement therapy that reduces the infection; however, overuse of antibiotics has been directly associated with the development of microbial resistance and decreased outcomes.<sup>32</sup> Therefore, other approaches need to be investigated.<sup>33</sup>

The most common reason for impaired wound healing is wound infection.<sup>34</sup> Wound infection is caused here is an imbalance exist between the microorganism and the immune barriers of the host. For this reason, microbiologic counts of the wound tissue are a useful guide to the degree of wound contamination and the potential for wound repair. If bacterial counts exceed  $10^5$  organisms per gram of tissue, healing is adversely affected.<sup>34,35</sup> Treatments need to be investigated that may positively affect wound healing.

## Procedures

We used a computer generated randomization table to assign eighty Sprague-Dawley rats into one of four groups (20 per group): Group 1, (Ceftriaxone); Group 2 (UVL light only); Group 3, (UVL light plus Ceftriaxone); or Group 4 (no treatment). The rats were fed antibiotic-free rat chow provided by a Bridge Point approved vendor; the animals were provided tap water ad libitum. The rats were allowed to drink water up to the point of the experiment because they cannot vomit and do not have the risk of aspiration. The rats were housed in individual polycarbonate boxes (24cm X 45cm and 21cm height). The bedding was provided by a Bridge Point approved vendor. The rats were identified by cage cards for each animal. The animals were housed and cared for in accordance with the guideline "Guide for the care and Use of laboratory Animals" approved by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). During the initial acclimation period of six days, the rats received a comprehensive veterinary health inspection to ensure the health of the animals prior to the commencement of the study. The general health and appearance of the experimental animal were monitored prior to the experiment.

The rats were anesthetized by injection of a mixture of Ketamine, (6.06 mg/Kg); Acepromazine (0.30 mg/Kg); and Zylazine 6.67 mg/Kg. The inoculum for this study was *staphylococcus aureus* obtained from frozen cultures. Three days prior to the experiment, a small loopful of test bacteria was streaked from the frozen culture collection and cultured on a nonselective agar plate. The bacteria were incubated overnight at  $37\text{ degrees C} \pm 1\text{ degree}$ . The culture was



examined visually for purity prior to use. In the pure culture state, all of the bacterial colonies demonstrated the same morphology and color. The day prior to the day of inoculation, a small loopful of the bacteria was collected from the 24 hour agar plate into a 7mL of diluent in a sterile test tube. The bacteria was incubated at  $37^{\circ}\text{C} \pm 1$  degree with shaking at 150 rpm overnight

After anesthetization, we clipped the skin over the dorsal cervical region, and skin was prepared with 70% isopropyl alcohol. One incision was immediately distal to the skull with the incision perpendicular to the spine. Each incision was 2 cm in length. A small subcutaneous pocket was produced along the length of the incision. On the morning of infection, the investigators prepared the organisms to a density of approximately  $10^7$  colony-forming units per milliliter (CFU/mL) in a phosphate buffer saline. Specifically, the bacteria suspension was centrifuged at 8000 rpm (ThermoScientific, FiberLife, Chicago) for 15 minutes. Overnight cultures were grown to a density of  $10^8$  CFUs/mL. The supernatant was removed and the pellet was suspended in 7 mL of diluent. A solution of 0.1 mL of the bacterial. Copa Foam that was inoculated with *staphylococcus aureus* was inserted into the space created. The wounds were stapled and the edges were adhered together with a sterile glue. After surgery, we returned the rats to their individual clean cages. Groups 1 and 3 were administered Ceftriaxone 100 mg/Kg on day 0 and day 1. For the subjects in the UVL groups, the light was used on day 3, 5, 10, and 14. All groups that received UVL received the light one inch from the wounded site at 180 seconds of exposure. Group 4 served as a negative control and did not receive any treatment. The technicians, staff, and investigators wore UVL-protecting face shield and goggles to prevent eye damage during treatments. Microbiological samples were taken immediately after the UVL treatment.

For pain management, we administered buprenorphine at 0.02mg/100 grams. The microbiological sampling consisted of removing the implant. The biopsy tissue was placed into a pre-weighed vessel containing 0.5 mL of diluent. The biopsy samples were individually homogenized and serially diluted. The serial dilutions were drop plated and incubated to determine the bacterial counts. The counts are expressed as CFU/g log to the tenth power. The individual analyzing the counts was blinded to group assignment.

The tissue above (dorsal to) the implant was carefully removed from the pocket. The biopsy tissue was placed into a pre-weighed vessel containing 0.5mL of sterile solution, and the weight of tissue was determined. The biopsy samples were individually homogenized and serially diluted. The serial dilutions were drop plated and incubated to determine the bacterial counts. The bacterial counts were expressed as  $\log_{10}$  (CFU/g)

## Results

The rats were weighed before the start of the study. By use of Analysis of Variance (ANOVA), we found that there were no significant differences in weight of the rats by group indicating that the groups were equivalent on this variable ( $p > 0.05$ ). The means and standard deviations were calculated for the bacteria count and are summarized in table 1. The zero count does not mean that there were no bacteria but they were not detectable. The results suggest that the Ceftriaxone group was more effective on day three compared to all the other groups.

**Table 1: Bacterial count by group and by day expressed in a log 10 CFUs/g**

<b>Group</b>	<b>Day</b>	<b>Mean <math>\pm</math> Standard Deviation</b>
Ceftriaxone	Three	$0 \pm 0$
	Five	$1.22 \pm 1.59$
	Ten	$1.31 \pm 1.72$
	Fourteen	$3.41 \pm .569$
UVL	Three	$5.40 \pm 1.03$
	Five	$5.24 \pm .817$
	Ten	$4.26 \pm 1.46$
	Fourteen	$3.16 \pm 1.89$
Ceftriaxone + UVL	Three	$1.07 \pm 1.73$
	Five	$5.24 \pm 1.74$
	Ten	$0.00 \pm 000$
	Fourteen	$3.50 \pm .378$
No Treatment	Three	$1.07 \pm 1.39$
	Five	$2.00 \pm 1.43$
	Ten	$3.328 \pm 2.28$
	Fourteen	$4.01 \pm .553$

A Repeated ANOVA showed that there were significant differences over time among the groups ( $p < 0.05$ ). The data are summarized in Table 2.

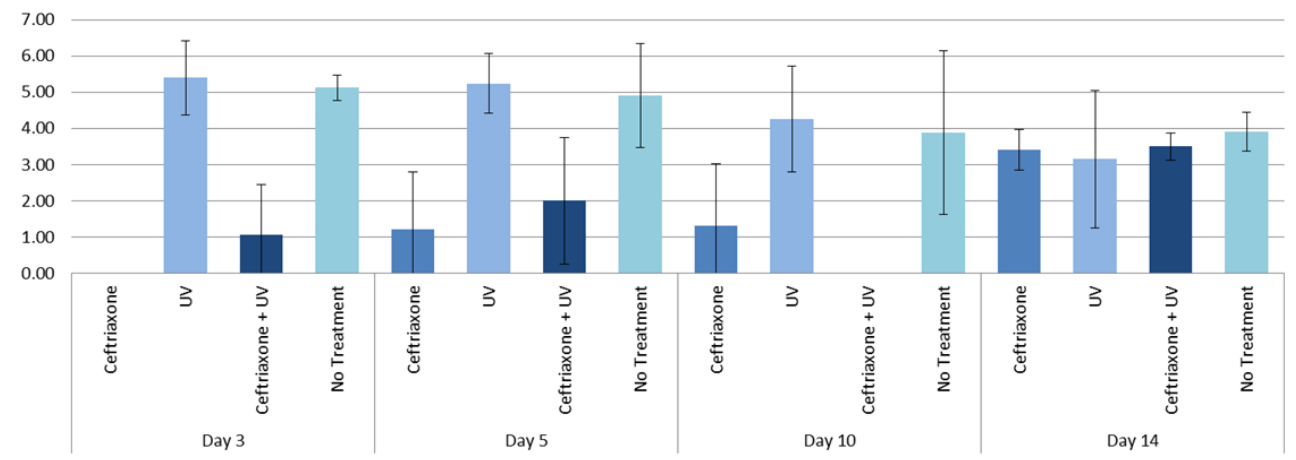
**Table 2 Differences in bacteria count by group and day**

<b>Day</b>	<b>Group</b>	<b>P value</b>
Three	Ceftriaxone less than UVL	*.000
	Ceftriaxone less than Ceftriaxone + UVL	*.012
	Ceftriaxone less No Treatment	*.000
Five	UVL no difference from No Treatment	.430
	Ceftriaxone less than UVL	*.000
	Ceftriaxone no difference from Ceftriaxone + UVL	.232
Ten	Ceftriaxone less than No Treatment	*.000
	UVL no difference than No Treatment	.614
	Ceftriaxone less than UVL	*.000
Fourteen	Ceftriaxone no difference from Ceftriaxone + UVL	.063
	Ceftriaxone less No Treatment	*.000
	UVL no different than No Treatment	.206
Fourteen	Ceftriaxone no difference than UVL	.257
	Ceftriaxone no difference than Ceftriaxone + UVL	.851
	Ceftriaxone less No Treatment	.247
	UVL no difference from No Treatment	.101

\*Significant at the 0.05 level

The difference in total bacteria count are summarized by group and day in Figure 1

**Figure 1: Log CFUs of total bacteria by group and time**



### Significance of the Current Study Relative to Other Studies

Ultraviolet light has an electromagnetic spectrum that greater in energy than visible light. Germicidal UVL has equal to or greater than 254 nanometers and is mutagenic and lethal to microorganisms.<sup>36</sup> The light causes damage to the nucleic acid of microorganisms by forming covalent bonds between adjacent thymine bases in the deoxyribonucleic acid (DNA).<sup>37,38</sup> Because of the formation of strong covalent bonds prevents, the DNA cannot replicate preventing the microorganism from reproducing.<sup>39</sup> Several *in-vitro* studies have found the effectiveness of UVL for the control of microbes in controlled settings.<sup>40-43</sup> Dai et al.<sup>43</sup> showed a 3-5 log reduction of cell inactivation when fungal suspensions were exposed to limited germicidal irradiation. Riley et al.<sup>44</sup> found similar results with dental implants coated with *Escherichia coli*. In that study, bacteria were killed at a rate of 650 million per cm<sup>2</sup>. However, *in vivo* studies demonstrating improved wound healing from the use of germicidal light therapy are limited and represent a void of scientific knowledge regarding both a potential bactericidal effect and/or an improved wound healing benefit and are urgently needed to determine the efficacy of such treatment in the management of infections. Therefore, this study investigated the use of a therapy to reduce or prevent wound related infections, which potentially improve wound healing. We speculated that if the UVL therapy worked, it could be a potential for the treatment of wound infections in deployed or austere environments. This is one of the first studies to investigate the use of UVL and compare to the use of an antibiotic.

Most of the research relative to UVL has been related to disinfection of food, water, supplies and equipment. Most of the studies indicate that the use of UVL is effective.<sup>45-54</sup> As an example, a recent article reviewed the effectiveness of UVL and hydrogen peroxide for terminal room decontamination and concluded that multiple studies show that the combination of UVL and hydrogen peroxide inactivate microbes and decontaminate surfaces in hospital rooms naturally contaminated with multidrug-resistant pathogens.<sup>55</sup> Likewise, Zakaria et al found that UVL was effective for surface disinfection in emergency toilets.<sup>54</sup>

Few studies have investigated the effects of UVL on tissue and infection. Dujowich et al. found that low-dose UVL showed promise as a rapid, effective, and synergistic means of reducing bacterial counts including *staphylococcus aureus* in canine skin and muscle.<sup>56</sup> Gupta et al. found that UVL at 2000-280 nm was highly effective relative to antimicrobial and acute wound infections to kill pathogens without host tissue damage. They concluded that UVL into tissue is limited and may damage the DNA in the host. They emphasized that the risk has to be balanced against the beneficial effects. They further point out that exposure to UVL may be carcinogenic. They concluded that more research is needed with both animal and human models.<sup>57</sup> Ledon, et al. developed a systematic review of the effects of UVL therapy on onychomycosis and concluded that the therapy may be effective; however, the review did not include studies relative to wound infection.<sup>58</sup> Likewise, Ortiz et al. reviewed the use of laser and UVL for the treatment of onychomycosis and concluded that the treatment was effective.<sup>59</sup> Mu et al. investigated the antibacterial nanoparticles including UVL and concluded they were effective in inhibiting the formation of pathogens including both gram-positive and gram-negative organisms.<sup>60</sup> Rhodes et al. investigated the use of UVL against *Escherichia coli* and concluded that UVL using 405 nm eradicated antibiotic resistant gram-negative bacteria.<sup>61</sup> Wu found that there was longer survival of alloskin grafts *in situ* by pretreating the alloskin samples grafted with both an anti-beta 2-microglobulin monoclonal antibody and irradiation with UVL light.<sup>62</sup>

### **Significance of Study to Military Nursing**

The results of this study indicates that the use of UVL was not effective in treatment of infection in mice. The results indicate the antibiotics such as Ceftriaxone need to be implemented early in the course of an infection. The data also show that the antibiotic need to continue because there were no significant differences in microorganism counts and all group had an increase from day 3 through 10.

### **Effect of problems or obstacles on the results:**

There were numerous problems and obstacles relative to this study. The investigator contacted the Triservice Research Facility, Southwest Research, Institutional Surgical Research, and the University of Texas Health Science Center. The last three charged between 46%-70% indirects, which was not budgeted. In addition, the latter three required that a local Principal Investigator (PI) be appointed with salary. The staff at Triservice Research Facility also required a local PI with no salary; however, none of the staff members was interested in the study. All of the facilities thought that the study was underfunded. We contacted Bridge Point, and they were able to implement the study. They also believed study to be underfunded and would only implement the first experiment. Another problem was that one of the animals died while being anesthetized but this did not change the results of the study.

### **Limitations**

All of the staff and personnel dealing with rats including the microbiologist who conducted the microorganism count were blinded to the study. In addition, meticulous procedures were used and monitored for consistency. Although the procedures were followed the same for each group,

the study had limitations. The major limitation of the study was that it was underfunded and could not fund all parts of the proposed study. We speculated that because the data show that UVL was not effective in treating infections that it had little or no effect on wound repair. The study also used only one type of UVL light and only one antibiotic. The longer use of UVL light and other types with antibiotics may yield different results. Another limitation of the study was that we used a mouse model that may not translate to humans.

## Conclusion

In conclusion, the data from this study indicates that this particular UVL was not effective in treatment of infection in mice. The results indicate the antibiotics need to be implemented early in the course of an infection. The data also show that the antibiotic need to continue because there were no significant differences in microorganism counts once the treatment was terminated. The microbial count rose to pretreatment levels. Other types and intensities of UVL need to be investigated along with other antibiotics.

## Changes in Clinical Practice, Leadership, Management, Education, Policy, and/or Military Doctrine that Resulted from Study or Project

None at this time

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**Summary of Dissemination**

<b>Type of Dissemination</b>	<b>Citation</b>	<b>Date and Source of Approval for Public Release</b>
Publications	None at this time. A query letter was sent to several journals relative to the study. None of the journals was interested in submission of a manuscript.	
Publications in Press	None at this time. See above	
Published Abstracts	None at this time	
Podium Presentations	None at this time	
Poster Presentations	None at this time	
Media Reports	None at this time	
Other		

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**Reportable Outcomes**

<b>Reportable Outcome</b>	<b>Detailed Description</b>
Applied for Patent	None
Issued a Patent	None
Developed a cell line	None
Developed a tissue or serum repository	None
Developed a data registry	None

**Recruitment and Retention Table**

<b>Recruitment and Retention Aspect</b>	<b>Number</b>
Animals Projected in Grant Application	80
Animals Purchased	80
Model Development Animals	5
Research Animals	
Animals With Complete Data	79
Animals with Incomplete Data	

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<b>Recruitment and Retention Aspect</b>	<b>Number</b>
Animals Projected in Grant Application	80
Animals Purchased	80
Model Development Animals	
Animals Intervention	20 per group

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Principal Investigator: Johnson, Arthur Don Col (ret)

USU Project Number: N09-P03